Marked up version of the abstract at page 88

Abstract Of The Invention

A method of treating or preventing development of a neurological disorder has been developed wherein a subject with the disorder, or at risk of developing a disorder, is vaccinated against a brain protein or antigen. Alternatively, the antibodies can be directly administered to the individual in need of treatment thereof. Animal studies demonstrate potent efficacy in the treatment of epilepsy, stroke and cognition in animal models vaccinated against the NMDA receptor.

Marked-up version of the title at page 1

[VACCINE-MEDIATED TREATMENT OF NEUROLOGICAL DISORDERS] NMDA VACCINE FOR THE TREATMENT OF EPILEPSY

Marked up version of paragraph 5 at page 8

Fig. 7A is a graph showing the effect of vaccination of the behavior of rats in a line crossing test. Data represents the number of line crossings in 5 min intervals over 5 successive days in AAVlac-treated animals (squares-solid line) or AAVNMDAR1-vaccinated animals [(diamond-dashed line)] (triangle-solid line);

Marked up copy of the first paragraph at page 47

[Neurobasal™] NEUROBASAL™ [medium] serum-free basal medium containing B27 supplement and 0.5 mM L-glutamine (all from Gibco BRL). Medium was replenished every 48 h, with the addition of a mitotic inhibitor (0.5 μM cytosine arabinoside) after 4 days. Cultures were grown for at least 9 days prior to calcium imaging.

Marked up version of paragraph 2 at page 49

For ß-galactosidase antibody screening, 1 µg purified ß-galactosidase protein (Sigma) was separated on a 10% acrylamide gel under reducing conditions and transferred to a nitrocellulose membrane. Serum samples from AAVNMDAR1, naïve and AAVlac animals (1:200), or monoclonal ß-galactosidase (1:5000, Gibco BRL) were applied for 1 h at room temperature (RT) or overnight at 4°C following a 90 min incubation in Tris-buffered saline containing 0.1% Tween 20 (TBST) containing 5% fetal calf serum (FCS) to block non-specific binding. Bound antibodies were detected using a peroxidase-labeled anti-rat or mouse antibody (1:12,000, Sigma) for 1 h at RT, and visualized using the ECL detection system (Amersham). Hippocampal and cortical extracts were prepared from naïve rat brain. Two preparations were used: (i) a crude hippocampal or cortex extract was prepared by homogenizing the tissue in ice cold 320 mM sucrose in 10 mM Tris-HCl, pH 7.4; (ii) a non-denatured membrane extract was prepared by homogenizing tissue as described above, in the presence of protease inhibitors ([Mini Complete™,] MINI COMPLETE™, protease inhibitor Boehringer Mannheim). Following centrifugation at 7000g, 10 min, 4°C, the resulting supernatant was centrifuged at 37,000 g, 40 min, 4°C and the pellet resuspended in 10 mM Tris-HCl, pH 7.4 containing protease inhibitors. For NMDAR1 antibody screening, 20 µg total hippocampal extract was separated on a 12% reducing gel or 20 µg non-denatured hippocampal membrane protein on a 10%

Marked-up version of the paragraph 2 at page 66

For the circular track mobility test, the track used was a modified version of one used to test mobility in mice (Carlsson *et al.* (1990) *Life Sci.* 47: 1729). Each rat was placed inside the track at the start position, facing clockwise, and the number of circuits completed in 5 minutes was recorded. This procedure was conducted for 5 days. Fig. 7A depicts the results from the line crossing test and Fig. 7B depicts the results from the circular track mobility test. Data represents the number of line crossings in 5 min intervals over 5 successive days in AAVlac (squares-solid line) or AAVNMDAR1 rats [(diamond-dashed line)] (triangle-solid line). In the circular track test, the number of completed circuits in successive days for AAVlac (n=6) and AAVNMDAR1 (n=6) animals are represented.

Marked-up claims showing revisions

1. (Amended) A method for treating a subject with a neurological disorder, or at risk of developing a neurological disorder comprising:

administering a vaccine comprising a therapeutically effective amount of an antigen, wherein the antigen elicits the production of antibodies in the circulatory system of the subject, or a composition comprising a therapeutically effective amount of an isolated antibody, or an antibody portion, wherein the antibodies bind to, and modify the function of a target protein in the central nervous system, to thereby [ameliorate or prevent the onset of] treat a neurological disorder in the subject.

3. (Amended) The method of claim 1, wherein the disorder is selected from the group consisting of epilepsy, stroke, Alzheimer's disease, Parkinson's disease, dementia, Huntington's disease, amyloid lateral sclerosis and depression.

7. (Amended) The method of claim 6, wherein the antigen is an [NMDA] N-methyl-D-aspartate (NMDA) receptor.

8. (Amended) The method of claim 7, wherein the antigen is [NMDAR1] N-methyl-D-aspartate receptor subunit 1 (NMDAR1).

26. (Amended) The method of claim 25, wherein the antigen is selected from the group consisting of an [NMDA] N-methyl-D-aspartate (NMDA) receptor, a [GluR] glutamate receptor (GluR), an [NPY] neuropeptide Y (NPY), galanin, an [NK-1] neurokinin-1 receptor (NK-1), a dopamine transporter and glutamic acid decarboxylase.

27. (Amended) The method of claim 26, wherein the antigen is an [NMDA] N-methyl-D-aspartate (NMDA) receptor.

28. (Amended) The method of claim 27, wherein the antigen is [NMDAR1] N-methyl-D-aspartate receptor subunit 1 (NMDAR1).

39. (Amended) The method of claim 38, wherein the antigen is an [NMDA] N-methyl-D-aspartate (NMDA) receptor.

40. (Amended) The method of claim 39, wherein the antigen is [NMDAR1] N-methyl-D-aspartate receptor subunit 1 (NMDAR1).

46. (Amended) The method of claim 45, wherein the target protein is an [NMDA] N-methyl-D-aspartate (NMDA) receptor.

54. (Amended) A method for treating a subject with a neuroendocrine disorder, or at the risk of developing a neuroendocrine disorder comprising:

administering a vaccine comprising a therapeutically effective amount of an antigen to a subject, wherein the antigen elicits the production of antibodies in the circulatory system of the subject, or a composition comprising a therapeutically effective amount of an isolated antibody, or an antibody portion, wherein the antibodies bind to, and modifies the function of a target protein in the central nervous system, to thereby [ameliorate the neuroendocrine disorder, or to prevent the onset of] treat the neuroendocrine disorder in the subject.

70. (Amended) A [pharmaceutical] composition comprising a therapeutically effective amount of an antigen capable of eliciting the production of antibodies in the circulatory system of the subject, or a therapeutically effective amount of an isolated antibody, or an antibody portion, wherein the antibodies bind to, and modify the function of a target protein in the central nervous system.

71. (Amended) The [pharmaceutical] composition of claim 70, wherein antibodies pass across the blood-brain barrier into the central nervous system facilitated by injury, disease or excessive neuronal activity.

72. (Amended) The [pharmaceutical] composition of claim 71, wherein the antigen selected from the group of neurotransmitters, neuroreceptors, transporters, ion channels, signal transduction molecules, enzymes involved in the synthesis or degradation of neurotransmitters, growth factors, transcription factors and cell surface molecules.

73. (Amended) The [pharmaceutical] composition of claim 72, wherein the antigen is an [NMDA] N-methyl-D-aspartate (NMDA) receptor.

74. (Amended) The [pharmaceutical] composition of claim 73, wherein the antigen is [NMDAR1] N-methyl-D-aspartate receptor subunit 1 (NMDAR1).

75. (Amended) The [pharmaceutical] composition of claim 70, wherein the target protein is selected from the group of neurotransmitters, neuroreceptors, transporters, ion channels, signal transduction molecules, enzymes involved in the synthesis or degradation of neurotransmitters, growth factors and transcription factors.

76. (Amended) The [pharmaceutical] composition of claim 75, wherein the target protein is an NMDA receptor.

REMARKS

An amendment and response to the Office Action is submitted in which the Examiner's rejections have been considered. Claims 1-76 are pending in the application. Claims 1, 3, 7, 8, 26, 27, 28, 39, 40, 46, 54, and 70-76 have been amended as suggested by the Examiner. No new matter has been added.

Amendment of the claims should in no way be construed as an acquiescence to any of the Examiner's rejections and was done solely to more particularly point out and distinctly claim the invention to expedite the prosecution of the application. Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s).

Formalities

As requested in the Office Action, the specification has been amended to correct a typographical error in the Abstract, provide a new title and correctly identify trademarked reagents. The Examiner is thanked for the helpful suggestions.

In paragraph 4b, the Office Action states that there is no description of Figs 2E-2H. In response, applicant submits that as indicated on the Figures, the left panel (i.e., Figs 2A, 2C, 2E, and 2G) are different cell types that are identified using antibodies for particular markers that are present on the cell type. Fig 2A, are SIRP myeloid cells, Fig 2C are dendritic cells, Fig 2E are helper T cells/macrophages, and Fig 2G are monocytes/macrophages. The right panel (i.e., Figs 2B, 2D, 2F and 2H) are each of the different cells types that also show expression and co-localization of the NMDAR1 protein in each cell type, determined by using antibodies against the NMDAR1 protein, as described at page 55, line 17 through page 56, line 8 of the specification.

Similarly in paragraph 4c, the Office Action notes that the bars in Fig. 6 are not labeled. Applicant has submitted formal drawings in which the bars in Fig. 6 are labeled.

In paragraph 4d, it is asserted that the Drawings in Figs. 7A-7B cannot be differentiated because both lines are solid. Applicant has submitted formal drawings in which the lines are labeled. The squares-solid lines are AAVlac-treated animals, while the triangles-solid lines are AAVNMDAR1-treated animals.

In paragraph 4e, the Office Action asserts that in Figs 12A-12D, it cannot be determined which lines and bars represent each particular rat group. Applicant has submitted formal drawings in which the graphs and bars are labeled.

Drawings

Applicant submits formal drawings, enclosed herein.

Claim Objections

Claim 3 was objected to for failing to recite the word "disease" after "Alzheimer's and Parkinson's". Accordingly, Claim 3 has been amended to insert the word "disease" after Alzheimer's and Parkinson's, thereby rendering the objection moot.

Rejection of Claims 1-3, 5-12, 22-32, 36-46, 54, 59-61, 68, and 70-76 Under 35 U.S.C. § 112, First Paragraph

Claims 1-3, 5-12, 22-32, 36-46, 54, 59-61, 68, and 70-76 have been rejected under 35 U.S.C. § 112, first paragraph. In particular, the Examiner asserts that:

> [t]he specification does not teach effective antigen administration, such as quantity or route or administration, to achieve the desired production of antibodies. Further, the working examples with the genetic vaccine do not provide guidance regarding how to administer the protein vaccine. the specification does not disclose any methods or working examples directed to the treatment neurological and endocrine disorders with the antigen vaccine. the specification provides no guidance or working examples directed to the modification of a target protein or to the improvement of cognition with the antigen vaccine.

Applicant respectfully traverses the rejection. To fulfill the enablement requirement under 35 U.S.C §112, first paragraph, the specification must describe how to make and use the claimed invention. However, it is well known that enablement is not precluded by the necessity for some experimentation (see, e.g., *In re Wands* 8 USPQ2d 1400-1407, 1404 (CAFC, 1988)). Furthermore, "the specification need not contain an example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *In re Borkowski*, 422 F.2d 904, 908, 164 USPQ 642, 645 (CCPA 1970)" *See* MPEP 2164.02.

The pending claims are drawn to a method for treating a subject with a neurological disorder, or at risk of developing a neurological disorder by administering a vaccine comprising a therapeutically effective amount of an antigen that elicits the production of

antibodies in the circulatory system of the subject, or a composition of a therapeutically effective amount of an isolated antibody, or an antibody portion. The antibodies bind to, and modify the function of a target protein in the central nervous system, to ameliorate or prevent the onset of, or treat a neurological disorder in the subject.

Other pending claims are drawn to methods of modifying the functions of target proteins in the central nervous system, by administrating an antigen vaccine, as well as to methods for improving cognition, and methods of treating neuroendocrine disorders.

Contrary to the Examiner's assertion, the specification provides sufficient guidance to the skilled artisan on how to select an appropriate antigen that is capable of eliciting antibodies, and how to administer such antigens. For example, at page 57, line 17 through page 58, line 30, the specification describes how to make peptides of the NMDAR1 receptor, and how to determine whether these peptides are an epitope for the NMDAR1 antibody by epitope mapping experiments. This is accomplished by generating 94 peptides that are 16 amino acids in length and which overlap by 6 amino acid residues. The specification describes how to identify which NMDAR peptides are actual epitopes for NMDAR antibody by using the serum from rats that had previously been vaccinated with either AAVNMDAR1, AAVlac or naïve rats, to screen against this panel of 16mer peptides (*See* pages 57-58). The results shown at page 57 of the specification and in Figs 3D-3H, and demonstrate that

> [n]one of the naïve (n=4) or AAVlac rats (n=14) screened had specific binding to any of the peptides. In contrast, AAVNMDAR1-immunized rats showed specific binding to peptides which corresponded to functional domains within the extracellular segments of the receptor. These included peptide 49 (amino acids 483-498) which represented the N-terminal side of M1, the first transmembrane domain (rat N19, Fig. 3E), and two peptides corresponding to the extracellular domain between M3 and M4, peptide 69 (amino acids 681-696; rat N21, Fig. 3F) and peptide 72 (amino acids 711-726; rat N64, Fig. 3H). Each of these three peptides contain critical residues for glycine binding (Kuryatov *et al* (1994) *Neuron* 12: 1291; Wafford *et al.* (1995) *Mol. Pharmacol.* 47: 374; Wood *et al.* (1997) *J. Biol. Chem.* 272: 3532). The most common pattern observed in serum from 7 of the 19 AAVNMDAR1 rats

> screened was specific binding to peptides adjacent to the M4n region (peptide 80, amino acids 791-807) and/or peptide 65 (amino acids 641-657) corresponding to the M3c domain (rat N11, Fig. 3D). Two additional rats had antibodies that bound to peptides 54/55 (amino acids 541-566; rat N52, Fig. 3G) which mapped to the preM1 domain. The preM1, M4n and M3c regions form part of the extracellular vestibule of the NMDA receptor channel where amino acid substitutions at key residues have a significant influence on channel permeability (Beck *et al.* (1999) *Neuron* 22: 559).

From the data presented, the skilled artisan would conclude that the epitopes for NMDAR1 is peptide 65 (amino acids 641-657), peptide 49 (amino acids 483-498), peptide 69 (amino acids 681-696), peptide 55 (amino acids 541-566), peptide 72 (amino acids 711-726), and peptide 80 (amino acids 791-807). The specification also indicates how these amino acids correspond to the overall structure of the NMDAR1 receptor. For example, peptide 49 represents the N-terminal side and the first transmembrane domain, while peptides 69 and 72 correspond to the extracellular domain, and that "each of these three peptides contains critical residues for glycine binding." Applicant also identifies a number of other epitopes, e.g., peptides 80, 65 and 55, which map to different regions of the receptor, such as the M4n region, the Mc3 region and the preM1 region, respectively. In addition, Applicant provides guidance on where the skilled artisan can obtain more information about the different regions of the NMDAR receptor by making reference to a number of citations that refer to the different structural sites of the NMDAR1 receptor. For example, at page 58, lines 17-22 of the specification:

> [I]ndividual animals had antibodies which bound to 16mers that mapped to the extracellular vestibule of the channel including the preM1, M3c, M4n domains (Beck *et al.* (1999). *Neuron* 22: 559), as well as epitopes in the N terminal region and within the extracellular loop lying between M3 and M4 which directly mapped to glycine binding sites (Kuryatov *et al.* (1994) *Neuron* 12, 1291; Wafford *et al.* (1995) *Mol. Pharmacol.* 47, 374; Wood, *et al.* (1997) *J. Biol. Chem.* 272, 3532).

Furthermore, the specification provides ample guidance on how to make and use a peptide vaccine. For example, in terms of quantity, applicant states that the dose and effective amount can be determined based on the characteristics of the active compound, i.e, the peptide, and provides a non-limiting range of about 0.1-20 mg/kg, more preferably 1-10mg/kg. The skilled artisan will appreciate that these doses will vary according to the size, sex and weight of the subject (*See* page 30, lines 3-25).

Applicant describes the administering the antigen peptide by coupling the peptide to a carrier such as keyhole limpet hemocyanin (KLH) and human serum albumin. Applicant describes a means for conjugating the peptide to suitable carriers, and provides a list of a number of suitable carriers at page 33 of the specification as follows:

> [A]lternatively, the antigen can be administered as a peptide vaccine. A synthetic peptide comprising an antigen binding region can be prepared using standard peptide synthesis method known in the art. It is often necessary to couple the peptide with a carrier. Exemplary and preferred carriers are keyhole limpet hemocyanin (KLH) and human serum albumin. Other carriers may include a variety of lymphokines and adjuvants such as INF, IL2, IL4, IL8 and others. Means for conjugating a peptide to a carrier protein are well known in the art and include glutaraldehyde, m-maleimidobenzoyl-N-hydroxysuccinimide ester, carbodiimyde and bis-biazotized benzidine. It is also understood that the peptide may be conjugated to a protein by genetic engineering techniques that are well known in the art. The preparation of vaccines which contain peptide sequences as active ingredients is generally well understood in the art. (*See e.g.*, U.S. Pat. Nos. 4,608,251; 4,601,903; 4,599,231; 4,599,230; 4,596,792; and 4,578,770).

The specification describes that the preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). Particularly by intramuscular or subcutaneous injection (*See* page 27, lines 11-15). the specification also describes a number of ways that the peptide vaccine can be formulated for administration, for example at page 26, line 22 though page 30, line 25, and more specifically at page 26, line 22 through page 27, line 24.

Accordingly, Applicant has provided ample guidance to the skilled artisan to select one or more epitopes of NMDAR, and generate a peptides that corresponds to the epitopes. Applicant has described that the peptide can be conjugated to KLH, or serum albumin, both of which are common carriers that are known to the skilled artisan. Furthermore, applicant has taught how a number of administration methods. Thus, from the guidance provided, the skilled artisan would be able to generate a peptide antigen and use it as a vaccine in a subject.

Moreover, the state of the art at the time the application was filed was such that peptides of NMDAR1, or fusion proteins that contained peptides of NMDAR1, were prepared to generate antibodies. Manufacture of antibodies using peptides fragments of proteins was a routine procedure that one skilled in the art would have knowledge of at the time of filing as indicated by a representative number of reference abstracts.

(1) Luo *et al* (1996) *Brain Res Dev Brain Res* 92:10-7

Describe a polyclonal antiserum to a fusion protein corresponding to a region of the NMDAR1 (NR1) subunit (amino acids 656-811) that was produced and affinity purified.

(2) Benke *et al* (1995) *J Recept Signal Transduct Res* 15:393-411

Describe the immunobiochemical characterization of the NMDA-receptor subunit NR1 in the developing and adult rat brain. In particular, two polyclonal antisera [NR1(N) and NR1(C)] were raised against fusion proteins derived from the N- and C-terminal domain of the NR1-subunit, respectively to investigate the developmental and regional expression of the NR1-subunit of the NMDA-receptor at the protein level.

(3) Siegel et al (1994) Proc Natl Acad Sci U S A 91:564-8

Describe the regional, cellular, and subcellular distributions of N-methyl-D-aspartate (NMDA) receptor subunit 1, NMDAR-1, in monkey hippocampus by using a monoclonal antibody directed against a fusion protein corresponding to amino acids 660-811 of NMDAR-1.

Using peptide vaccines to modify the diseases states was also been documented as shown by a representative few abstracts of references.

(1) Schenk *et al* (1999) *Nature* 400:173-177
Describe the attenuation of Alzheimer-disease using amyloid-beta peptide (Abeta)

(2) Morgan *et al* (2000) *Nature* 408:982-985
Show that amyloid beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. In particular, vaccinations with amyloid-beta peptide (A beta) dramatically reduce amyloid deposition in a transgenic mouse model of Alzheimer's disease.

(3) Janus *et al* (2000) *Nature* 408:979-982
Show that amyloid beta peptide immunization reduces behavioral impairment and plaques in a model of Alzheimer's disease.

Thus, in view of the knowledge available in the art and with the guidance provided by applicant in the specification, a skilled artisan would be able to make and use the claimed invention without undue experimentation.

With regard to the lack of working example, under MPEP 2164.02, "the specification need not contain an example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *In re Borkowski*, 422 F.2d 904, 908, 164 USPQ 642, 645 (CCPA 1970)" *See* MPEP 2164.02.

It is Applicant's position that there is sufficient guidance to enable one skilled in the art to practice the invention without undue experimentation.

With regard to claims 70-76, the examiner asserts that:

[t]he specification does not teach how to use a "pharmaceutical" composition comprising the NMDAR1 antigen . . .(Note, the issue could be overcome by deleting the word "pharmaceutical" form the claims).

In response, applicants have amended claims 70-76 to delete the word "pharmaceutical", thereby rendering the rejection moot.

Rejection of Claims 1-3, 5-12, 22-32, 36-46, 54, 59-61, 68, 73-74 and 76 Under 35 U.S.C. §112, Second Paragraph

Claims 1-3, 5-12, 22-32, 36-46, 54, 59-61, 68, 73-74 and 76 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In particular, the Examiner asserted that

> [C]laims 1-3, 5-12, 22-32, 36-46, 54, 59-61 and 68 are indefinite because the claims do not have a step that clearly relates back to the preamble.

Applicant respectfully traverses the rejection. Claims 22, and 36, and dependent claims thereof, clearly have a step that relates back to the preamble. For example, in claim 22, at page 79, line 16, the phrase "to thereby modify the function of the target protein" relates back to the preamble. Again, in claim 36, on page 81, line 17, the phrase "to thereby improve cognition of a subject" relates back to the preamble. Claims 1 and 54 have been amended to include the term "treat", the addition of which provides a step that relates back to the preamble. (It being understood that the term "treat" is used in its comprehensive sense, to either ameliorate or prevent the onset of neurological disorder.) In view of the amendments to the claims 1 and 54, the claims clearly have steps that relates back to the preamble. Accordingly, the Examiner is respectfully requested to withdraw the rejection.

The Examiner also asserts that in claims 7, 26-27, 39, 46, 73, and 76, "the acronym 'NMDA' renders the claims vague and indefinite".

In response, applicant has amended claims 7, 26-27, 39, 46, 73, and 76 to include the spelled version of the acronyms in each claim, thereby rendering the rejection moot.

The Examiner further asserts that in claims 8, 28, 40, and 74, "the acronym 'NMDAR1' renders the claims vague and indefinite".

In response, applicant has amended claims 7, 26-27, 39, 46, 73, and 76 to include the spelled version of the acronyms in each claim, thereby rendering the rejection moot.

The Examiner further asserts that in claim 26, "the acronyms 'GluR', 'NPY' and 'NK-1' renders the claim vague and indefinite".

In response, applicant has amended claim 26 to include the spelled version of each acronyms, thereby rendering the rejection moot.

The Examiner further asserts that in claims 9, 29, 41 and 59,the phrase "or a combination thereof" renders the claims vague and indefinite.

Applicant respectfully traverses the rejection. The rejected claims drawn to a vaccine which can be a viral vector vaccine, a DNA vaccine, a peptide vaccine or a crude antigen vaccine alone, or in combination with each other. For example, the vaccine may be a peptide vaccine in combination with a crude antigen vaccine, or a viral vector vaccine with a crude antigen vaccine.

CONCLUSION

In summary, the above-identified patent application has been amended and reconsideration is respectfully requested for all the reasons set forth above. The Examiner is

urged to telephone the undersigned Attorney for Applicant in the event that such communication is deemed to expedite prosecution of this matter.

Respectfully submitted,

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